sequence-specific. Fuller did not achieve amplification of useful PCR products which are sequence-specific with the 40% glycerol or ethylene glycol as additives. By contrast, the inventors of the subject application discovered that 40% glycerol is actually detrimental for low temperature PCR with high polymerization specificity using moderately thermostable DNA polymerases; the inventors also unexpectedly found that the use of 10-20% glycerol with three DNA polymerases is effective for low temperature polymerase chain reactions.

The Examiner indicated that these arguments are not persuasive because the limitation "sequence-specific amplification products" is not in the claims. To that end, we have amended claims 1 and 9 above to recite that the claimed method produces sequence-specific amplification products. This, we believe, clearly distinguishes our invention from anything taught or suggested by either Fuller or Ruano. Fuller did not produce any sequence-specific amplification products, and Ruano is not concerned with low temperature cycle extension at all. Withdrawal of these rejections is believed to be order.

Claims 1-11 and 18-21 are rejected as anticipated under 35 U.S.C. §102(e) by Hong et al. (U.S. Patent No. 5,834,253) (the '253 patent). We first point out that the examiner seems to have misquoted or misinterpreted the Hong '253 patent. In column 19, lines 6-11, Hong et al. mentioned using 50% glycerol as a preservative for the Bst DNA polymerase, <u>not</u> 10% (w/v) for reaction; in column 12, lines 14-24, Hong et al. mentioned using a DNA polymerase selected from Bacillus stearothermophilus for DNA sequencing, <u>not</u> for cycle amplification of a template; and in column 12, lines 56-64, Hong et al. mentioned using a method comprising nucleotides analogs, such as ddNTPs, dITP for sequencing reactions, <u>not</u> for cycle amplification of a template.

It is true that the Hong '253 patent mentioned using a DNA polymerase which would have homology (99-100%) to the instantly claimed SEQ ID Nos. 1-4, but the polymerase is for the purpose of DNA sequencing, <u>not</u> for cycle amplification.

In column 11, lines 59-65 and column 18, lines 60-62, the '253 patent mentioned using a method to extend a DNA primer for DNA sequencing, not in "repeated cycle primer extension" which is key for cycle amplification such as in the presently claimed invention. The same can be said of the other citations made by the Examiner: column 20,

lines 13-21; column 16, lines 60-67; column 17, lines 1-3; column 18, lines 55-67; and column 19, lines 1-45. In all these examples cited in the '253 patent, no glycerol is included as a component of the reaction mixture although 50% glycerol is used as the preservative of the concentrated DNA polymerase. The quantity of glycerol which is contained in the latter enzyme as a preservative and which may be carried over to the reaction mixture is negligible and cannot function as a melting agent for repeated cycle primer extension.

Whereas the '253 patent does not relate to (or subsequently teach) cycle extension (e.g., repeated cycles), our claims are directed to methods for extending an oligonucleotide primer or a pair of oligonucleotide primers via repeated cycles and affirmatively recite that the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product. This is believed to clearly distinguish our invention from the Hong '253 patent. Withdrawal of this rejection is believed to be in order.

Claims 22-35 are rejected as anticipated under 35 U.S.C. §102(e) by Hong et al. (U.S. Patent No. 6,165,765) (the '765 patent). As support for this rejection, the Examiner cited from the '765 patent column 8, lines 5-7; column 10, lines 42-57; column 15, lines 50-67; column 16, lines 1-16, column 23, lines 3-21; column 6, lines 48-64 and column 6, lines 48-64. However, all of these quotations are concerned with the teachings of using a modified or unmodified DNA polymerase for ***a single, non-repetitive, non-cycle primer extension*** in the reaction mixture ***without glycerol added as a melting agent for the purpose of DNA sequencing***. A 5% and 50% of glycerol (column 23, lines 3-31) were used in the isolation and purification of DNA polymerase, not as a melting agent in an enzymatic reaction. This is in contrast to our claimed invention, which is directed to a method for extending an oligonucleotide primer or a pair of oligonucleotide primers via repeated cycles. Independent claims 22 and 30 affirmatively recite that the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product.

As to the Examiner's reference to the '765 patent regarding the use of a DNA polymerase having homology (99-100%) to the instantly claimed SEQ ID Nos. 1-4, and the Examiner's citation of column 7, lines 1-23 regarding claim 29, we again emphasize

that the polymerase referred to in the '765 patent is used for DNA sequencing with a single non-cycle extension of the molecules of a primer to different lengths in the presence of a chain terminator, namely a dye-labeled dideoxynucleotide. The same DNA polymerase may be used in our subject invention, but for different purpose in different technology under different conditions. This should not render our claims anticipated.

Reconsideration of this rejection is also requested.

Lastly, claims 22-35 are rejected as anticipated under 35 U.S.C. §102(e) by Hong et al. (U.S. Patent No. 6,485,909) (the '909 patent). These claims are also rejected under the doctrine of obvious-type double-patenting as unpatentable over Hong et al. (U.S. Patent No. 6,485,909). As with the '253 patent and '765 patent, the same arguments can be presented with the '909 patent. The basic problem is that there was no cycle extension (repeated cycles) involved in these prior Hong patents. If glycerol was used, it was used for the purpose of preservation of an enzyme, not in the concentration of the instant claims and not for the purpose of lowering the melting temperature of a double-stranded DNA for the purpose of repeated cycle amplification of a primer or a pair of primers. Our claimed invention is directed to methods for extending an oligonucleotide primer or a pair of oligonucleotide primers via repeated cycles. Independent claims 22 and 30 affirmatively recite that the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product. Our claims are neither anticipated nor rendered obvious by the '909 patent. Withdrawal of these rejections is also requested.

In summary, then, someone having ordinary skill in this art, and having in hand any of Fuller, Ruano, the Hong '253 patent, the Hong '765 patent, or the Hong '909 patent, would not have reasonably found our invention described therein.

All of the Examiner's outstanding rejections and objections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly solicited. No amendment made was related to the statutory requirements of patentability unless expressly stated herein, and no amendment made was for the purpose of narrowing the scope of any claim unless we argued above that such amendment was made to distinguish over a particular reference or combination of references.

If the Examiner has any questions or would like to make suggestions as to claim language, she is encouraged to contact Marlana K. Titus at (301) 762-8214.

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MARKED-UP VERSION OF CLAIMS AS AMENDED ABOVE

1. (Twice Amended) A method for extending an oligonucleotide primer or a pair of oligonucleotide primers using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature of about 70°C, comprising the step of mixing a template DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template, in a solution containing between about 10% and about 20% (v/v) glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C and an annealing temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product.

9. (Amended) A method for extending an oligonucleotide primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature below about 80°C, comprising the steps of:

(i) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein

the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template, a suitable concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, and

(ii) effecting cycle primer extension reaction(s) at a temperature below about 80°C for a sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs and thereby produce a sequence-specific amplification product.

22. (Amended) A method for extending an oligonucleotide primer or a pair of oligonucleotide primers using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature of about 70°C, comprising the step of mixing a template DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase during dye-labeled terminator automated DNA cycle sequencing reduces the innate selective discrimination against the incorporation of fluorescent dye-labeled ddCTP and fluorescent dye-labeled ddATP, in a solution containing between about 10% and about 20% (v/v) glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C and an annealing temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product.

30. (Amended) A method for extending an oligonucleotide primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature below about 80°C, comprising the steps of:

(iii) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase during dye-labeled terminator automated DNA cycle sequencing reduces the innate selective discrimination against the incorporation of fluorescent dye-labeled ddCTP and fluorescent dye-labeled ddATP, a suitable concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, and

(iv) affecting cycle primer extension reaction(s) at a temperature below 80°C for a sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs <u>and thereby produce a sequence-specific amplification product</u>.